Gene Therapy with Galectin-3 Inhibits Bronchial Obstruction and Inflammation in Antigen-challenged Rats through Interleukin-5 Gene Downregulation

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The pathophysiology of asthma involves an intricate network of molecular and cellular interactions. Elevated Th2 cytokines (interleukin [IL]-5 and IL-4) associated with eosinophilic inflammation characterize allergic diseases and provide potential targets for immunomodulation. Recent evidence has demonstrated that galectin-3 induces selective downregulation of IL-5 gene expression in several cell types (eosinophils, T cell lines, and antigen specific T cells). Accordingly, we sought to elucidate whether *in vivo* intratracheal instillation of plasmid DNA encoding galectin-3 would inhibit an experimental asthmatic reaction in a rat model with increased eosinophils and T cells in bronchoalveolar fluid and impaired pulmonary function. We found that instillation of galectin-3 gene in these rats led to normalization of the eosinophil and T cell count in bronchoalveolar lavage fluid and that there was a strong concomitant inhibition of IL-5 mRNA in the lungs. As a consequence, galectin-3–treated rats showed recovery of pulmonary functional parameters, such as pulmonary pressure and expiratory flows. These data emphasize the potential utility of galectin-3 as a novel therapeutic approach for treatment of allergic asthma.

Keywords: gene therapy for asthma; interleukin-5 gene downregulation; galectin-3; eosinophilic airway inflammation

Asthma is a chronic inflammatory disorder of the airway, with infiltration of mast cells, eosinophils, and lymphocytes and reversible airflow limitation. This disease develops with episodes of wheezing, coughing, shortness of breath, and airway hyperresponsiveness to nonspecific stimuli. Eosinophilic inflammation is characterized by eosinophil accumulation in the airways, caused by a release of interleukin (IL)-5 by Th2 lymphocytes and other cells (1, 2). This IL is the main growth, differentiation, and survival factor for eosinophils and, indeed, is essential for initiation of allergen-induced eosinophilic airway inflammation (3, 4).

The severity of asthma is linked to the degree of airway eosinophilia (5). In humans, eosinophils may contribute to the pathogenesis of asthma by releasing a number of inflammatory mediators and toxic products, including oxygen and nitrogen radicals (6) and cationic proteins, which can severely damage the airway epithelium and increase airway reactivity.

The role of IL-5 in this disease would seem to make it a good target for asthma therapy. Using systemic administration of anti–IL-5 monoclonal antibodies, which inhibit antigen-induced airway eosinophilia in experimental models, several approaches have shown that eosinophilic inflammation and airway hyperresponsiveness may be prevented (7, 8).

Galectin-3 (Gal-3; an immunoglobulin [Ig] E-binding protein) belongs to a family of proteins that bind β-galactosides. It has a unique amino-terminal domain, a highly conserved repetitive sequence rich in proline and glycine, and a globular carboxyl-terminal domain containing the carbohydrate recognition site. Gal-3 is expressed in a variety of tissues and cell types (9). This protein is localized mainly in the cytoplasm, although significant amounts can also be detected in the nucleus, on the cell surface, and in the extracellular environment (10). Gal-3 has been implicated in different processes, including inflammation and allergic pathologies (11, 12). Recently, we reported that Gal-3 induces selective downregulation of IL-5 gene expression (13). As a consequence, there is a decrease in IL-5 secretion in different cell types (human eosinophils, EoL-3 cells, peripheral blood mononuclear cells, and antigen-specific T cell line [CD4+] derived from an allergic patient). This primary effect on IL-5 gene raises interesting possibilities in the regulation of allergic reactions (10, 13).

This study sought to examine further the role of Gal-3 in an *in vivo* model. We used Brown-Norway rats, a well-characterized model with several inflammatory and immunologic features resembling those of asthma, that is, airway eosinophilic inflammation, development of airway hyperreactivity, elevated IgE serum levels, and expression of Th2 cytokines (14).

Our results demonstrate that intratracheal instillation of plasmid-Gal-3 (pGal-3) in sensitized and antigen-challenged rats by inhalation leads to an improvement, not only regarding the cellular inflammatory infiltrate, but also in pulmonary function. There is normalization in both eosinophil and T cell numbers and a strong inhibition of IL-5 mRNA in the lungs of treated rats. These results suggest a novel therapeutic approach for asthma treatment.

METHODS

Experimental Protocol

Sixty male Brown-Norway rats weighing 300 g were used. Sensitization with ovalbumin (OVA) was performed as previously described (15). Fourteen days later, rats were treated, using orotracheal instillation, with 0.5 ml of plasmid (1 mg/ml) with (*1*) Gal-3 (OVA+plasmid enhanced green fluorescence protein [pEGFP]−Gal-3), (*2*) antisense Gal-3 (OVA+pEGFP−AS), and (*3*) without insert (OVA+pEGFP) or with saline instead of plasmid instillation (OVA) as positive control. Thereafter, until Day 19, rats were exposed to aerosolized 1% OVA solution for 15 minutes per day. On Day 20, functional tests were performed on all rats. An additional negative control group was used, in which rats were injected with saline and exposed to saline inhalation without plasmid treatment (SS).

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Plasmid DNA Preparation

Gal-3 gene (a plasmid gift from Dr. F.-T. Liu, La Jolla Institute for Allergy and Immunology, San Diego, CA) was cloned into an eukaryotic expression vector pEGFP plasmid (Clontech, Palo Alto, CA). Empty pEGFP plasmid and pEGFP–antisense Gal-3 were used as controls. pDNA was purified using the Wizard DNA purification system (Promega, Madison, WI) and stored at −20°C in pyrogen-free saline.

Gal-3 Expression in Lung by Immunoblotting and Polymerase Chain Reaction

Lung was homogenized, and immunoblotting was performed as described elsewhere (10). Polymerase chain reaction (PCR) to Gal-3 was performed on 5–10 μl of DNA product in a volume of 50 μl. The primers were designed in accordance with a published sequence (16).

Bronchoalveolar Lavage, Cell Analysis, and RNA Preparation

Cells obtained by bronchoalveolar lavage (BAL; 5 ml of saline for three washes) were counted and used for RNA extraction and for cytometric analysis. Briefly, 1×10^6 cells/ml were incubated for 30 minutes with a saturating concentration of fluorescein isothiocyanate-labeled anti-rat CD3 (G4.18), anti-rat granulocytes (HIS48), or anti-rat CD49d (integrin α4β1) to detect T cells, granulocytes, and eosinophils, respectively. The monoclonal antibodies were purchased from PharMingen (San Diego, CA) and were analyzed using a flow cytometer (Epics XLMCL; Coulter, Hialeah, FL).

Total RNA was isolated from BAL cells and lungs by an RNeasy kit (Qiagen, Chatsworth, CA) and were treated with Dnase I (Promega). RNA was measured by spectrophotometry, and 0.5–1 μg was used for the first-strand cDNA synthesis with avian myeloblastosis virus (AMV) reverse transcriptase (Promega).

Real-Time Quantitative PCR to IL-5

Primers were designed from a published sequence, and amplified product was 298 bp (17). We used LightCycler-FastStart DNA Master SYBR Green I for PCR. Samples were normalized at 0.5 μg of RNA free of DNA. Samples were denatured at 95°C for 10 minutes and amplified for 40 cycles at 95°C for 15 seconds, 50°C for 10 seconds, and 72°C for 15 seconds in a LightCycler system (Roche Diagnostics, Mannheim, Germany).

Determination of Total Serum IgE and Specific Antibodies

Total IgE levels were determined as described (18). All reagents were a gift from Professor H. Bazin (University of Louvain, Belgium). Specific IgE and IgG anti-OVA as well as IgG antidiphtheria were determined by enzyme-linked immunosorbent assay.

Lung Function

Rats were anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneally), tracheotomized, artificially ventilated, and paralyzed with pancuronium bromide (1 mg/kg, intraperitoneally). The tracheal pressure at end inspiration of the tidal volume breathing, pulmonary pressure, was measured as previously described (15). The pulmonary pressure registered after inspiratory capacity determination was considered for the comparison among the groups. Inspiratory capacity was defined as the change in lung volume between airway pressure 0 and 30 cm H_2O.

Forced expiration maneuvers were performed by inflating the lung to 30 cm H_2O and rapidly deflating to residual volume with a negative pressure of −40 cm H_2O. Forced vital capacity (FVC) and expiratory flow at 75% of FVC (F75) were derived directly from the expiratory flow-volume curves as previously described (15, 19).

Statistical Analysis

Data from multiple experiments are expressed as mean ± SD, and statistical significance was determined using an unpaired *t* test, with $p < 0.05$ taken as significant.

RESULTS

Detection of Gal-3 in Lung Tissue

We set out to study the expression of the encoded protein in lung tissue. Immunoblotting of lung lysates was performed using anti–Gal-3 monoclonal antibody. In Figure 1A, Gal-3 was strongly detected in the lung of rats, which received pGal-3 (Lanes 5 and 6). The other groups, which had not received plasmid with Gal-3 (SS, OA, pC), presented a minimal constitutive expression of Gal-3. Recombinant Gal-3 was used as a positive control (Lane 1). Lane 2 (SS) corresponded to lung lysates from SS group, Lane 3 (OA) to lysates from OVA group, and Lane 4 (pC) to lysates from pEGFP group.

These data were confirmed by PCR, and similar results were obtained. Figure 1B demonstrates that the Gal-3 signal is easily detected in lung lysates from rats treated with pGal-3. In Figure 1B, C+ represents a positive control plasmid with Gal-3. Thus, PCR and Western blot analyses clearly indicate that Gal-3 is overexpressed in lungs from rats treated with pEGFP-Gal-3.

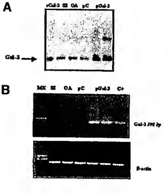

Figure 1. Gal-3 expression. (*A*) Ten micrograms of total lung protein from different groups of treated rats were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by Western blot stained with anti–Gal-3 monoclonal antibody. Lane 1 represents recombinant Gal-3 as positive control. Lane 2 (SS) represents lung lysates from SS. Lane 3 (OA) represents lysates from OVA. Lane 4 (pC) represents lysates from pEGFP, and Lanes 5 and 6 (pGal-3) represent lysates from pEGFP-Gal-3. (*B*) Gal-3 amplification by PCR in the same samples. Groups are identical as in *A*. Lane 7 (C+) represents a positive control plasmid with Gal-3. β-Actin was used as normalized control.

Effect of pGal-3 on Quantitative Expression of IL-5 Gene in BAL Cells

The effect of Gal-3 overexpression on the IL-5 gene was analyzed by quantitative PCR in cells from BAL. In the SS group, the mean IL-5 level was 0.3 ± 0.15 pg of DNA (Figure 2), against which the OVA group registered a 40-fold increase in IL-5 level, with a mean concentration of 12.02 ± 3.2 pg ($p < 0.05$). The administration of pEGFP failed to modify IL-5 mRNA expression in OVA-exposed animals (Figure 2). However, the mean level of IL-5 decreased to 1.4 ± 1.1 pg in OVA-exposed rats treated with pGal-3 (OVA+pEGFP+Gal-3), representing a

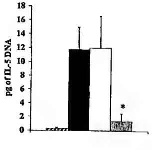

Figure 2. Quantitation of IL-5 gene expression in lung from different groups of rats (*see text*). mRNA was isolated from lungs, and cDNA specific to IL-5 was determined by real-time quantitative RT-PCR. Results shown are mean ± SD of three independent experiments. Type of standard curve analysis used was second derivative maximum with r = −1.00, and mean squared error 0.15. Differences are statistically significant (*$p < 0.05$) between OVA+pEGFP−Gal-3 versus OVA and OVA+pEGFP (SS = *hatched bars*; OVA+pEGFP = *black bars*; OVA = *white bars*; OVA+pEGFP−Gal-3 = *gray bars*).

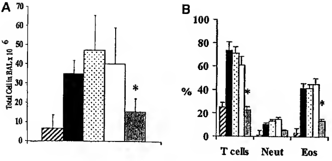

Figure 3. (*A*) The total cell counts in the BAL of rats from different groups (*see text*) are shown. Results are expressed as the mean ± SD of total cells harvested determined by light microscopy. SS (n = 8), OVA (n = 15), pEGFP–AS (n = 10), and pEGFP–Gal-3 (n = 17) (*p < 0.01 versus OVA). (*B*) Differential cell population counts in the BAL. Results are expressed as the mean ± SD of the percentage of the different cell types measured by flow cytometry (SS = *hatched bars*; OVA+pEGFP = *black bars*; OVA+pEGFP–AS = *dotted bars*; OVA = *white bars*; OVA+pEGFP–Gal-3 = *gray bars*).

90.7% reduction vis-à-vis the OVA-treated group. Data shown in Figure 2 are the mean ± SEM of n = 4 rats from each group ($p < 0.05$).

Administration of pGal-3 Prevents Cellular Infiltration of Airways

Cellular airway inflammation is a pivotal event in allergen-induced airway sensitization, and as expected, the downregulation of the IL-5 gene produced changes in the composition of cells from the BAL. Figure 3A shows that after sensitization and airway challenge with OVA and instillation with empty plasmid (OVA+pEGFP) or antisense plasmid (OVA+pEGFP–AS, n = 10), the absolute number of BAL cells registered a significant increase compared with nonsensitized (SS) rats ($35.24 \pm 9 \times 10^6$ and $47.66 \pm 19 \times 10^6$ cells versus $6.7 \pm 0.9 \times 10^6$ cells, n = 8). In contrast, in the OVA+pEGFP–Gal-3–treated group (n = 17), we observed a significant decrease in the total BAL cell number ($15.57 \pm 7 \times 10^6$ cells) as against the OVA group ($40.40 \pm 20 \times 10^6$ cells, n = 15, $p < 0.001$).

The percentage of different cell populations in BAL was also examined. The main cell populations found in OVA-sensitized rats were T cells and eosinophils, although some neutrophils (1–13%) were observed in BAL from all groups. Treatment with pGal-3 (OVA+pEGFP+Gal-3) altered the cell profile of OVA-sensitized rats, resulting in a significant reduction in eosinophils (69.6% decrease) and T cells (62.4% decrease) in BAL fluid ($p < 0.005$; Figure 3B). No differences were found in total cell number and cell populations when OVA+empty plasmid (OVA+pEGFP) and OVA antisense Gal-3 (OVA+pEGFP–AS) were compared. These results indicate that administration of pEGFP-Gal-3 in lung modulates allergen-induced BAL eosinophilia and T cell accumulation.

Levels of Total Serum IgE and Specific Antibodies

We wished to ascertain whether treatment with plasmid-encoding Gal-3 might alter total and specific IgE levels. OVA-sensitized rats had high levels of total and specific serum IgE. As can be seen in Table 1, there were no detectable changes in IgE from sensitized rats treated with plasmid with Gal-3 and OVA+empty plasmid. Data in Table 1 indicate that Gal-3 interferes neither with total nor with specific IgE synthesis.

Determination of specific IgG antibodies against immunizing agents showed that neither the response against OVA nor that against diphtheria was modified in treated rats. Antibody titers were similar across all sensitized groups (data not shown).

Pulmonary Function Recovery after Intratracheal Instillation of pGal-3

We were also interested in ascertaining whether the observed improvement in eosinophilic and T cell inflammation reflected a recovery of lung function. Figure 4 shows the pulmonary pressure in all four groups of OVA-sensitized rats. Pulmonary pressure was quantified in terms of the percentage change compared with the SS group (n = 8), considered as 100%. A significant increase in pulmonary pressure was found in the OVA (n = 10), OVA+pEGFP–AS (n = 10), and OVA+pEGFP (n = 10) groups, namely, 147.83 ± 7.58%, 134.30 ± 7%, and 150.4 ± 8.2%, respectively. In contrast, the pulmonary pressure of the OVA+pEGFP–Gal-3 group (n = 17) decreased toward control level (118.18 ± 6.31%), thus proving significantly different from the OVA group ($p < 0.005$).

In Figure 5, FVC and expiratory flow after F75 are expressed as the percentage of decline with respect to the SS group. FVC and F75 were significantly decreased in both the OVA, OVA+pEGFP–AS, and OVA+pEGFP groups vis-à-vis the SS group. In contrast, rats receiving Gal-3 partially returned to control values, with both F75 and FVC being significantly different from that of the OVA group ($p < 0.005$).

TABLE 1. OVALBUMIN-SPECIFIC ANTIBODY AND TOTAL IMMUNOGLOBULIN E LEVELS IN THE SERUM OF CONTROL AND TREATED RATS

Rat Group	OVA-specific IgE (*UA/ml*)	Total IgE Levels (*μg/ml*)
Saline	125 ± 22	22.2 ± 8
OVA	2,627 ± 200*	90.7 ± 40*
OVA+pEGFP	2,705 ± 73*	100 ± 31*
OVA+pEGFP–Gal-3	2,787 ± 70*	110 ± 32*

Definition of abbreviations: Gal-3 = galectin-3; IgE = immunoglobulin E; OVA = ovalbumin; pEGFP = plasmid enhanced green fluorescence protein.

Serum titers for OVA-specific antibodies and total IgE were determined by enzyme-linked immunosorbent assay. Data presented are mean ± SD.

* p < 0.05 versus nonsensitized group.

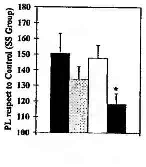

Figure 4. Pulmonary pressure percentage with respect to control (SS group). Pulmonary pressure increment in OVA, OVA+pEGFP–AS, and OVA+pEGFP–Gal-3 expressed as percentage compared with control group, taking the control value as 100% and not represented in this figure (for details, *see text*). *Pulmonary pressure in OVA+pEGFP–Gal-3 was significantly decreased vis-à-vis the OVA (p < 0.005) (OVA+pEGFP = *black bars*; OVA+pEGFP–AS = *dotted bars*; OVA = *white bars*; OVA+pEGFP–Gal-3 = *gray bars*).

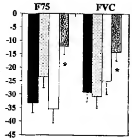

Figure 5. F75 and FVC reduction percentage with respect to control values. FVC (*right panel*) and expiratory flow after F75 (*left panel*) reduction expressed in percentage with respect to control group, considered zero and not represented in this figure (for details, *see text*). *Decline in both F75 and FVC was significantly less in the OVA+pEGFP−Gal-3 than in the OVA ($p < 0.005$) (OVA+pEGFP = *black bars*; OVA+pEGFP−AS = *dotted bars*; OVA = *white bars*; OVA+pEGFP−Gal-3 = *gray bars*).

DISCUSSION

Airway inflammation in asthma is a complex phenomenon that is predominantly driven by Th2-type cells. An understanding of the molecular mechanisms regulating Th2 cytokine production *in vivo* is a key factor for development of novel therapies (20, 21).

The data presented here show that intratracheal deposition of a vector with the gene that codifies for Gal-3 in Brown-Norway rats causes a blunting of some Th2 effects locally in the lung (eosinophil influx and functional pulmonary parameters). Thus, Gal-3 plays an important role in the downregulation of IL-5 gene expression and the ensuing amount of eosinophils and T cells in BAL. As a consequence, there is an improvement in both the eosinophilic inflammation and functional respiratory parameters.

Gal-3 belongs to a family of soluble proteins with affinity for β-galactose–containing oligosacharides. In a previous study, we demonstrated that Gal-3 downregulates IL-5 gene expression in different cell types, using CD32 (FcγRII) as receptor (10, 13). In addition, it probably works through inhibition of GATA-3, the main nuclear factor implicated in IL-5 gene regulation (22). Other authors have suggested a role for Gal-3 as a negative growth regulator (23) and as an inhibitor of the transcription of the granulocyte macrophage-colony-stimulating factor–driven responsive genes in rat mononuclear bone marrow cells, with an ability to suppress bone marrow cell proliferation *in vivo* (24).

IL-5 is one of the main regulatory cytokines that modulates eosinophils, the major inflammatory effector cells in allergic disorders (25, 26). Thus, IL-5 promotes eosinophil differentiation, CD34+ eosinophil progenitor mobilization from bone marrow cells, and eosinophil CCR3 expression in asthma; increases recruitment of eosinophils from circulation; prolongs survival of eosinophils; and primes eosinophils for degranulation and release of toxic metabolites (27–30). These effects make IL-5 an excellent target for treatment of airway eosinophilic inflammation (31).

As mentioned earlier, our previous studies *in vitro* indicated that Gal-3 induces downregulation of IL-5 gene in several cell types (10, 13). For this reason, we used Gal-3 in the treatment of antigen-specific airway inflammation in a rat model. Gene therapy may have some advantages over conventional pharmacologic treatment. First, it allows overexpression of Gal-3 in the target area intracellularly, its natural location; second, one is not limited by the amount of material. Finally, unlike other anti–IL-5 therapies, one avoids parenteral administration (32). Because the effect of Gal-3 is highly IL-5 selective, there are no collateral effects on other ILs. Hence, no other alterations to the immune response were to be expected in the treated animals. Direct gene transfer was used because it has been shown to result in rapid DNA uptake, and genes express a biologically active protein in lung (33). Naked plasmid administration is a simple and safe gene delivery method. Murine, macaque, and clinical human studies have demonstrated transfection of respiratory tissues after direct application of free plasmid. Furthermore, intratracheal administration of naked plasmid DNA led to transgene expression after 1 to 3 days and was detectable for as long as 28 days (34). Accordingly, we used a simple and safe alternative to lung transduction of Gal-3. Indeed, Gal-3 antiinflammatory effects were still in evidence for up to 30 days after gene transfer (data not shown).

Our results clearly show that administration of plasmid with Gal-3 gene decreases BAL eosinophil and T cell counts in OVA-challenged animals, due to IL-5 gene inhibition observed by quantitative PCR (Figures 2 and 3). IL-5 also induces vascular cell adhesion molecule-1 expression in endothelial cells, may promote eosinophil and lymphocyte migration by binding to its counterparts (very late antigen [VLA]-4 or αβ2 integrin), and promotes eosinophil survival (35, 36). Furthermore, IL-5 enhances eosinophil adhesion to bronchial epithelial cell (37). It is therefore not surprising that by decreasing IL-5 we decreased T cells and eosinophils in the airways.

The effect observed when using plasmid with Gal-3 gene is not due to nonspecific effects from naked plasmid, such as immunostimulatory DNA sequences, as antisense Gal-3 failed to produce any therapeutic effect.

Recent studies describe a diversification in the relationship between eosinophils, airway hyperresponsiveness, and T cells. This is dependent on several factors, for example, the animal model versus humans, and within the animal model itself, the specific strain and manner of immunization (38–41). All of these parameters must be taken in account when it comes to validating any given therapy, thereby rendering comparison between different strategies difficult. With respect to the results obtained with anti–IL-5 antibodies, it is important to point out that our model showed a decrease in both eosinophils and CD3+ T cells. This may explain why our rats underwent a significant recovery of pulmonary function in both expiratory flows and pulmonary pressures, not detected by anti–IL-5 humanized antibodies (32).

Recently, targeted disruption of the Gal-3 gene has been described as resulting in attenuated peritoneal inflammatory responses in thioglycolate-treated mice, caused mainly by lower numbers of macrophages. Furthermore, there were consistently more eosinophils in Gal-$3^{-/-}$ mice (42). According to our results, there is an inverse correlation between absence of Gal-3 and increased numbers of eosinophils. Total serum IgE level was not altered with pGal-3 treatment, in line with our previous data in which Gal-3 was shown to have no detectable effect on the IL-4 gene (13). Thus, other authors have demonstrated that whereas anti–IL-5 does prevent airway inflammation, anti-IgE does not (8). Other alternatives tested recently include the use of an IL-5 antisense oligonucleotide in mouse models of asthma, with inhibition of antigen-induced eosinophilia and late-phase airway hyperresponsiveness being reported (22). Recently, inhibition of antigen-induced eosinophilia and airway hyperresponsiveness has been reported, using antisense oligonucleotides directed against the common β chain of IL-3, IL-5, granulocyte macrophage-colony-stimulating factor receptors in a rat model of allergic asthma (43).

Whereas treatment with Gal-3–inhibiting IL-5 expression decreased eosinophil airway accumulation and prevented development of specific airway hyperresponsiveness, there was no interference with secretion of other cytokines, because IL-4, IL-2, and γ-interferon production in BAL were not modified with Gal-3 treatment (measured by PCR, data not shown). B cell immunoglobulin production was not altered because specific IgG

and IgE and total IgE serum levels were similar for all sensitized rats (whether or not treated with Gal-3).

Lung function evaluation shows that Gal-3 treatment produced a marked inhibition of the increase in pulmonary pressure and decrease in expiratory flows produced by OVA. Lung function studies covering these protocols usually tend to focus on lung resistance measurements (44, 45). These parameters are influenced not only by airway resistance, but also by airway secretion, parenchymal distortion, and tissue resistance (46). Indeed, in patients with asthma, spirometry and flow-volume curves are the reference techniques for determining the degree of airway obstruction. In this model, sensitization induced a 35% decrease in expiratory flows and a 50% increase in pulmonary pressure. To our knowledge, flow-volume curves have not been applied for the purpose of evaluating amelioration of bronchoconstriction in experimental rat models. The finding of a 60% inhibition of the OVA-induced decrease in both F75 and FVC could be regarded as truly remarkable.

Our ultimate goal is to devise new strategies for asthma treatment. Bronchodilators open the airways, and antihistamines and steroids reduce inflammation. However, by interrupting the chain of command that leads to the attack, researchers have identified an entire new set of promising therapies. Thus, cytokine gene delivery (47, 48) and immunostimulatory DNA sequences that inhibit Th2 response and enhance Th1 profile (45) are used as therapies in many clinical trials.

Given the predominant role of IL-5 in asthmatic reaction, inhibition of infiltration of eosinophils into the lungs inhibits much of the early sequelae of the disease. We used a novel treatment using plasmid encoding Gal-3 and obtained a marked inhibition of eosinophil airway accumulation and better lung function, thus opening up a new approach for future therapies.

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